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Applicant: Liu, *et al.* Examiner:
Serial No.: Art Unit:
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Title: Insect Control Agent

Assistant Commissioner for Patents
Washington, D.C. 20921

Sir:

PRELIMINARY AMENDMENT

On page 1, line before , please insert the following paragraph:

“This application is a continuation of U.S. Patent Application No. 09/448,256, which is incorporated by reference herein. This application claims the benefit of priority to U.S. Provisional Patent Application 60/164,078, which is also incorporated by reference herein.”

In the claims:

Please cancel claims 1 through 72.

Please add the new claims presented on the following pages.

09/22/01 - 09/22/01

73. An insect control agent comprising a baculovirus, wherein the baculovirus directs transcription of at least one ribonucleic acid (RNA) that, when present within an insect cell, forms a double-stranded structure that inhibits expression of at least one insect gene.

74. The agent of claim 73, wherein the RNA comprises two separate complementary strands.

75. The agent of claim 74, wherein the expression vector comprises a DNA segment flanked by two promoters, wherein the promoters are operably linked to the DNA segment, and wherein the promoters are oriented so as to direct transcription of both sense and antisense RNA transcripts from the flanked DNA segment.

76. The agent of claim 75 wherein the expression vector further comprises DNA sequences that direct termination of the RNA transcripts.

77. The agent of claim 75 or claim 76, wherein the expression vector further comprises at least one enhancer element operably linked to at least one of the promoters.

78. The agent of claim 73 wherein the RNA comprises one strand that is self-complementary.

79. The agent of claim 73 wherein at least a portion of the ribonucleic acid sequence is substantially identical to at least a portion of the sequence of the at least one gene.

80. The agent of claim 73, wherein the cell forms part of a tissue of a target pest organism.

81. The agent of claim 73, wherein the baculovirus is selected from the group consisting of: the *Autographa californica* multiple polyhedrosis virus, the *Orgyia pseudotsugata* MNPV, the *Lymantria dispar* MNPV, the *Helicoverpa zea* NPV, and the *Bombyx mori* NPV.

82. The agent of claim 73, wherein the at least one gene to be inhibited is an essential gene in the insect.

83. The agent of claim 73 wherein at least one gene to be inhibited is a gene involved in development in a pest organism.

84. The agent of claim 73, wherein at least one gene to be inhibited is involved in neurotransmission in a pest organism.

85. The agent of claim 73, wherein at least one gene to be inhibited is expressed in the insect alimentary canal or Malpighian tubules.

86. The agent of claim 73 wherein the at least one gene to be inhibited is naturally found in an insect selected from the order *Lepidoptera*.

87. The agent of claim 73 wherein the at least one gene to be inhibited is naturally found in an insect selected from the list consisting of: the cotton bollworm (*Helicoverpa zea*), the cabbage looper (*Trichoplusia ni*), the alfalfa looper (*Autographa californica*), the tobacco hornworm (*Manduca sexta*), the tobacco budworm (*Heliothis virescens*), the fall armyworm (*Spodoptera frugiperda*), the European corn borer (*Ostrinia nubilalis*), the eastern spruce budworm (*Choristoneura fumiferana*), the western spruce budworm (*C. occidentalis*), and the gypsy moth (*Lymantria dispar*).

88. The agent of claim 73, wherein the RNA is species specific in that it does not show more than approximately 80% homology with any known gene in any species other than the species in which the RNA sequence is naturally found.

89. The agent of claim 73, wherein the RNA is species specific in that it does not show more than approximately 80% homology with any known vertebrate gene.

90. An insecticidal composition comprising the agent of claim 73 and an agriculturally suitable carrier.

91. The composition of claim 90 further comprising at least one agent selected from the group consisting of: conventional pesticides, gustatory stimulants, thickening agents, UV screening agents, optical brighteners, viral synergists, dispersants, flow agents, spreading agents, and sticking agents.

92. A recombinant baculovirus comprising:

a first promoter;

a second promoter;

a DNA segment whose sequence comprises at least one sequence at least 80%

identical to a portion of the sequence of at least one insect gene, wherein the portion of the sequence of the at least one insect gene is at least 50 nucleotides in length;

a first enhancer operably linked to the first promoter,

a second enhancer operably linked to the second promoter,

a first transcriptional terminator, wherein the first transcriptional terminator is positioned so as to terminate transcription directed by the first promoter;

a second transcriptional terminator, wherein the second transcriptional terminator is positioned so as to terminate transcription directed by the second promoter, wherein the two promoters are operably linked to the DNA segment, and wherein the promoters are oriented so as to direct transcription of both sense and antisense RNA transcripts from the DNA segment.

93. The recombinant baculovirus of claim 92, wherein the at least one insect gene is selected from the list consisting of: genes that are essential in a pest organism, genes involved in neurotransmission in a pest organism, genes involved in development in a pest organism, and genes expressed in the insect alimentary canal or Malpighian tubules.

94. The recombinant insect virus of claim 92, wherein the DNA segment is species specific in that it does not show more than approximately 80% homology with any known gene in any species other than the species in which the gene is naturally found.

95. The recombinant insect virus of claim 94, wherein the DNA segment is species specific in that it does not show more than approximately 80% homology with any known vertebrate gene.

96. An composition comprising the recombinant insect virus of claim 92 and an agriculturally suitable carrier.

97. The composition of claim 96, further comprising at least one agent selected from the group consisting of: conventional pesticides, gustatory stimulants, thickening agents, UV screening agents, optical brighteners, viral synergists, dispersants, flow agents, spreading agents, and sticking agents.

98. A pest control agent comprising at least one recombinant baculovirus, wherein the at least one recombinant baculovirus directs transcription of a first ribonucleic acid (RNA) that, when present within an insect cell, hybridizes either with itself or with a second ribonucleic acid whose transcription is directed by the at least one recombinant baculovirus, thereby forming a double-stranded structure that inhibits expression of at least one gene expressed in the insect cell.

99. The pest control agent of claim 98, wherein the first ribonucleic acid hybridizes with itself, thereby forming a double-stranded structure that inhibits expression of at least one gene expressed in the insect cell.

100. The pest control agent of claim 98, wherein the first ribonucleic acid hybridizes with a second ribonucleic acid whose transcription is directed by the at least one recombinant baculovirus, thereby forming a double-stranded structure that inhibits expression of at least one gene expressed in the insect cell.

101. The pest control agent of any of claims 98, 99, or 100, wherein the gene is an endogenous gene.

102. The pest control agent of any of claims 98, 99, or 100, wherein the insect cell is contained in an insect.

103. The pest control agent of claim 102, wherein the insect is a *Lepidopteran*.

104. The pest control agent of claim 103, wherein the insect is selected from the group consisting of: the cotton bollworm (*Helicoverpa zea*), the cabbage looper (*Trichoplusia ni*), the alfalfa looper (*Autographa californica*), the tobacco hornworm (*Manduca sexta*), the tobacco budworm (*Heliothis virescens*), the fall armyworm (*Spodoptera frugiperda*), the European corn borer (*Ostrinia nubilalis*), the eastern spruce budworm (*Choristoneura fumiferana*), the western spruce budworm (*C. occidentalis*), and the gypsy moth (*Lymantria dispar*).

105. The pest control agent of any of claims 98, 99, or 100, wherein the insect cell is cultured *in vitro*.

106. The pest control agent of claim 105, wherein the insect cell is a *Lepidopteran* cell.

107. The pest control agent of any of claims 98, 99, and 100, wherein the gene is species specific in that it does not show more than approximately 80% homology with any known vertebrate gene.

108. The pest control agent of any of claims 98, 99, and 100, wherein the gene is found naturally in an insect of a first species, and wherein the gene is species specific in that it does not show more than approximately 80% homology with any known gene in any species other than the first species.

109. The pest control agent of any of claims 98, 99, and 100, wherein the gene is selected from the group consisting of: genes that are essential in a pest organism, genes involved in neurotransmission in a pest organism, genes involved in development in a pest organism, and genes expressed in the insect alimentary canal or Malpighian tubules.

110. The pest control agent of any of claims 98, 99, and 100, wherein the baculovirus is selected from the group consisting of: the *Autographa californica* multiple polyhedrosis virus, the *Orgyia pseudotsugata* MNPV, the *Lymantria dispar* MNPV, the *Helicoverpa zea* NPV, and the *Bombyx mori* NPV.

111. A pest control agent comprising an occlusion body containing a plurality of recombinant baculoviruses, wherein each of the recombinant baculoviruses directs transcription of a first ribonucleic acid (RNA) that, when present within an insect cell, hybridizes either with itself or with a second ribonucleic acid whose transcription is directed by at least one of the recombinant baculoviruses, thereby forming a double-stranded structure that inhibits expression of at least one gene expressed in the insect cell.

112. The pest control agent of claim 111, wherein the first ribonucleic acid hybridizes with itself, thereby forming a double-stranded structure that inhibits expression of at least one gene expressed in the insect cell.

113. The pest control agent of claim 112, wherein the first ribonucleic acid hybridizes with a second ribonucleic acid whose transcription is directed by the at least one recombinant baculovirus, thereby forming a double-stranded structure that inhibits expression of at least one gene expressed in the insect cell.

114. The pest control agent of any of claims 111, 112, or 113, wherein the gene is an endogenous gene.

115. The pest control agent of any of claims 111, 112, or 113, wherein the insect cell is contained in an insect.

116. The pest control agent of claim 115, wherein the insect is a *Lepidopteran*.

117. The pest control agent of claim 116, wherein the insect is selected from the group consisting of: the cotton bollworm (*Helicoverpa zea*), the cabbage looper (*Trichoplusia ni*), the alfalfa looper (*Autographa californica*), the tobacco hornworm (*Manduca sexta*), the tobacco budworm (*Heliothis virescens*), the fall armyworm (*Spodoptera frugiperda*), the European corn borer (*Ostrinia nubilalis*), the eastern spruce budworm (*Choristoneura fumiferana*), the western spruce budworm (*C. occidentalis*), and the gypsy moth (*Lymantria dispar*).

118. The pest control agent of any of claims 111, 112, or 113, wherein the insect cell is cultured *in vitro*.

119. The pest control agent of claim 118, wherein the insect cell is a *Lepidopteran* cell.

120. The pest control agent of any of claims 111, 112, and 113, wherein the gene is species specific in that it does not show more than approximately 80% homology with any known vertebrate gene.

121. The pest control agent of any of claims 111, 112, and 113, wherein the gene is found naturally in an insect of a first species, and wherein the gene is species specific in that it does not show more than approximately 80% homology with any known gene in any species other than first species.

. The pest control agent of any of claims 111, 112, and 113, wherein the gene is selected from group consisting of: genes that are essential in a pest organism, genes involved in transmission in a pest organism, genes involved in development in a pest organism, and as expressed in the insect alimentary canal or Malpighian tubules.

The pest control agent of any of claims 111, 112, and 113, wherein the baculovirus is selected from the group consisting of: the *Autographa californica* multiple polyhedrosis virus, *Orgyia pseudotsugata* MNPV, the *Lymantria dispar* MNPV, the *Helicoverpa zea* NPV, and the *Bombyx mori* NPV.

124. An insecticidal composition comprising the agent of claim 111 and an agriculturally suitable carrier.

125. The composition of claim 124 further comprising at least one agent selected from the group consisting of: conventional pesticides, gustatory stimulants, thickening agents, UV screening

agents, optical brighteners, viral synergists, dispersants, flow agents, spreading agents, and sticking agents.

126. A method of controlling insects, the method comprising the step of:

contacting a cell in an insect with a first ribonucleic acid (RNA) whose sequence corresponds to at least a portion of at least one gene endogenous to the insect, wherein the first ribonucleic acid hybridizes either with itself or with a second ribonucleic acid with which the cell is also contacted, thereby forming a double-stranded structure within the cell that inhibits expression of at least one gene expressed in the cell, .

1 The method of claim 126, wherein the first ribonucleic acid hybridizes with itself, thereby
f ng a double-stranded structure that inhibits expression of at least one gene expressed in the
i : cell.

1 The method of claim 126, wherein the first ribonucleic acid hybridizes with a second
r ucleic acid, thereby forming a double-stranded structure that inhibits expression of at least
c ene expressed in the insect cell.

1 The method of any of claims 126, 127, or 128, wherein the step of contacting with RNA
c rises introducing RNA into the cell or expressing RNA in the cell or both.

130. The method of any of claims 126, 127, or 128 wherein the step of contacting comprises contacting the insect with a baculovirus.

131. The method of claim 130, wherein the first RNA is expressed within 6 hours after the insect is contacted with the baculovirus.

132. The method of claim 130, wherein the RNA is expressed substantially in the absence of viral replication.

133. The method of claim 130, wherein the baculovirus does not establish a productive infection.

134. The method of claim 129, wherein the insect is a *Lepidopteran*.

135. The method of claim 134, wherein the insect is selected from the group consisting of: the cotton bollworm (*Helicoverpa zea*), the cabbage looper (*Trichoplusia ni*), the alfalfa looper (*Grapha californica*), the tobacco hornworm (*Manduca sexta*), the tobacco budworm (*liothis virescens*), the fall armyworm (*Spodoptera frugiperda*), the European corn borer (*trinia nubilalis*), the eastern spruce budworm (*Choristoneura fumiferana*), the western spruce worm (*C. occidentalis*), and the gypsy moth (*Lymantria dispar*).

The method of any of claims 126, 127, or 128, wherein the sequence of the gene portion is species specific in that it does not show more than approximately 80% homology with any known vertebrate gene.

i The method of any of claims 126, 127, or 128, wherein the gene is found naturally in an organism of a first species, and wherein the sequence of the gene portion is species specific in that it does not show more than approximately 80% homology with any known gene in any species other than the first species.

138. The method of any of claims 126, 127, and 128, wherein the gene is selected from the group consisting of: genes that are essential in a pest organism, genes involved in neurotransmission in a pest organism, genes involved in development in a pest organism, and genes expressed in the insect alimentary canal or Malpighian tubules.

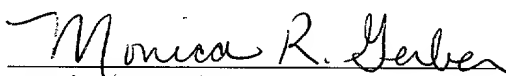
139. The method of claim 130, wherein the baculovirus is selected from the group consisting of: the *Autographa californica* multiple polyhedrosis virus, the *Orgyia pseudotsugata* MNPV, the *Lymantria dispar* MNPV, the *Helicoverpa zea* NPV, and the *Bombyx mori* NPV.

140. The method of claim 130, wherein the step of contacting comprises applying the baculovirus to organisms on which the insect feeds.

141. The method of any of claims 126, 127, or 128, whereby one or more biological or physiological functions of the insect is inhibited.

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Respectfully submitted,



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